Exhibit B

Additional Rat Data

Rat neural stem cells prepared as per Examples 1-5 in the application were utilised in the nuclear transfer procedures described in Example 12 of the application. The reconstructed eggs were cultured to the morula/blastocyst stage to determine the success of the nuclear transfer procedures. The results obtained are summarised in the following table:

Cell type	n	PN	2-cell	4-cell	Morula	Blastocysts
RFNSC**	158	109	72	11	5	0

** The data were based on 5 experiments.

PN represents the number of reconstructed embryos showing successful pronuclei formation.

RFNSC represent wild-type rat foetal neural stem cells;

The successful development of the reconstructed embryos beyond the 2-cell stage (at which point in the rat development becomes dependent on the embryonic genome as opposed to nutrients and proteins derived from the maternal oocyte) to the morula stage demonstrates that reprogramming of the neural stem cell nuclei enabled the expression of the relevant genes required to direct embryonic development. In the rat, therefore, neural stem cells represent a viable source of donor nuclei for nuclear transfer experiments.

Mouse Data

Mouse foetal neural stem cells were isolated and cultured essentially as described in Examples 1-5 of the application with the age and developmental time points of the foetus used adjusted accordingly. The isolation of mouse oocytes and subsequent nuclear transfer procedures are further described below:

Superovulation and Oocyte Collection of Mice

Four week old F1 female mice (CBA x C57B) were superovulated by intraperitoneal injection of 10 IU PMSG at 8:00 pm followed 48 hours later by 10 IU hCG. Treated female mice were killed by cervical dislocation, about 13 hours after hCG injection and the oviducts removed and transferred into a petri dish containing 3 ml HTF-HEPES medium supplemented with 300 IU/ml hyaluronidase. The oviduct ampullae were opened with fine watch-maker forceps and 30-gauge needle and the cumulus enclosed oocytes released. The dish was then placed on a warm plate at 37°C for 3-5 minute and as soon as the oocytes had separated

from cumulus cells they were recovered with a fine "hand-pulled" glass pipette and washed in two changes of embryo handling medium (HTF-Hepes). The oocytes were then placed in lots of approximately 30-40 in drop with 10 µl handling medium overlaid with mineral oil on the manipulation chamber.

Enucleation, Nucleus Injection, Activation and Culture

The enucleation of mouse oocytes was carried out 13-15 hrs after hCG injection by slitting the zona pellucida in the region of the cytoplasmic bulge using a microneedle with subsequent squashing of the metaphase plate through the slit with the holding pipette in Hepes-Buffer. The enucleated eggs were then placed in HTF embryo culture medium for 1 h before nuclear injection. Nuclei of mouse neural stem cell were injected into enucleated oocytes using pipettes drawn to an approximate inner diameter of 5 µm, which were back-loaded with mercury and coated with 5% polyvinylpyrrolidone (PVP) immediately before use. The pipette containing an isolated nucleus was put through the slit in the zona pellucida and advanced between half and three-quarters of the way through the oocyte. Piezoelectric actuation was used to break the membrane and the nucleus was deposited. The reconstructed eggs were then incubated for 10 min in Hepes at room temperature before being placed into the HTF embryo culture medium for 1 hour. Oocytes were then transferred to an activation medium containing Ca_2–free HTF-Hepes; 10 mM $SrCl_2$; and, 5µg/ml cytochalasin B for 6 hours. Following activation the eggs were washed twice with HTF embryo culture medium and cultured in 10mm wells containing 400 µl of culture medium overlaid with paraffin oil at 37°C in 5% CO_2 in air. The formation of a pronucleus was checked the next morning and the development of the eggs was recorded for 6 days. Cumulus cells freshly isolated from oocytes during the denuding (hyaluronidase treatment) step described above were also used as donor nuclei in order to compare the resulting development of the reconstructed eggs in vitro. Cloned mice have previously been reported using cumulus cells as the donor nuclei (Wakayama *et al*, 1998: Nature 394(6691): 369-74).

Results

The *in vitro* development results of the mouse nuclear transfer experiments described above are summarised in the following table:

Cell type	N	PN	2-Cell	4-cell	Morula	Blastocyst
Cumulus	182*	154	135	78	70	12
MFNSC	38**	28	10	4	3	3

*The data were based on three experiments.

** The data were based on 1 experiment.

PN represents the number of reconstructed embryos showing successful pronuclei formation.

MFNSC represent wild-type mouse foetal neural stem cells.

As for the rat above, the successful development of the reconstructed embryos beyond the 2-cell stage (at which point in the mouse development becomes dependent on the embryonic genome as opposed to nutrients and proteins derived from the maternal oocyte) to the morula and blastocyst stages demonstrates that reprogramming of the neural stem cell nuclei enabled the expression of the relevant genes required to direct embryonic development. In addition, although development to the morula stage was higher in embryos reconstructed with cumulus cells than those with neural stem cells, the development of embryos to the blastocyst stage at an equivalent rate in both groups suggests that such nuclei are similar in their ability to be reprogrammed and to direct embryonic development. As discussed above, embryos reconstructed using cumulus cell nuclei have previously resulted in live born pups. In the mouse, therefore, as for the rat, neural stem cells represent a viable source of donor nuclei for nuclear transfer experiments.